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IFCC Reference Methods for Measurement of pH, Gases and Electrolytes in Blood: Reference Materials[1])

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Summary: The Scientific Division Committee on pH, Blood Gases and Electrolytes of the International Federation of Clinical Chemistry (IFCC) produced recommendations to attempt to make the results of pH, blood gas and electrolyte analysis from different clinical chemistry laboratories internationally compatible.

The aim of this lecture is to discuss the essential aspects of

1. the IFCC approved (1986) reference method for pH measurement in blood,
2. the IFCC approved (1988) reference method for tonometry of blood,
3. the provisionally proposed recommendations on the expression of results obtained with ion-selective electrodes for measuring sodium, potassium and calcium in serum, plasma or blood and
4. the reference method for the determination of ionized calcium in serum, plasma or blood.

Also reference materials for quality control of pH, blood gas and electrolyte measurements are reviewed. Failures of several types of currently available quality control materials are discussed.

1. pH and Blood Gases

For the assessment of the state of the acid-base balance and gas transport, sophisticated instrumentation is available by which routine measurements of pH, pCO_2 and pO_2 can be made simultaneously in a small sample of blood. With the development of ion-selective electrodes, which enable measurement of the electrolytes sodium, potassium and ionized calcium directly in blood, these analysers now include blood gas/electrolytes analysers.

Although this instrumentation is well manufactured, the possibility of making great errors is not excluded e. g. a deviating residual liquid junction potential in the pH measurement. In addition the introduction of ion-selective electrodes for the measurements of the electrolytes gives problems because the results may be different from those obtained by flame photometry.

Often inadequate accuracy and precision lead to inadequate patient care. Errors can be fatal. To make results medically useful there is a need to establish the quality requirements of measurements.

The International Federation of Clinical Chemistry (IFCC)/Scientific Division Committee on pH, Blood Gases and Electrolytes, previously named the Expert Panel on pH and Blood Gases, started work in 1977 on basic documents dealing with the terms of reference:

1. definitions and terminology,
2. reference methods and
3. reference materials,

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to attempt to make the results of pH and blood gas analysis from different clinical chemistry laboratories internationally compatible.

In order for the measurement process of a quantity to be meaningful, a hierarchical system as diagrammed in figure 1 has been developed by scientists of the National Institute of Standards and Technology (NIST), previously the National Bureau of Standards (NBS) of the USA (1). This system indicates the coupling of the analytical methods (definitive method, reference method, field or routine method) and primary and secondary reference materials. The function of each of the components is to transfer *accuracy* to the level immediately below it and to provide *traceability* to the level immediately above it, thereby assuring compatibility in the overall measuring system.

I want to show you how this system may be applied to the establishment of the reference method for pH measurement in blood (2) and thereafter of the reference method for tonometry of blood (3).

1.1 pH

pH is *defined* as the negative logarithm to base 10 of the relative molal activity of hydrogen ions:

$$pH = - \lg a_{H^+}$$

The *definitive method* for pH measurement in dilute aqueous solutions is based on measuring the electromotive force of a cell with a hydrogen electrode and a silver/silver chloride electrode without a liquid-liquid junction i. e. without transference. This cell is often called the Harned cell.

The definitive method is employed by reference laboratories, e. g. the NIST, previously the NBS in USA, which has assigned pH(S) values to a series of *primary aqueous calibration solutions* in the pH range from 3 to 11 with an estimated uncertainty in pH of ± 0.005: the so called NBS-buffers.

Reference methods for pH measurement are based on the use of a cell with a hydrogen gas electrode and a reference electrode with a concentrated KCl liquid-liquid junction. Primary reference materials are used for calibration. These reference methods may be used to establish pH values of secondary calibration solutions.

The hydrogen gas electrode is unsuitable for pH measurements in biological fluids due to protein contamination of the platinum surface, where the present IFCC reference method for pH measurement in blood should be based on the glass electrode.

The IFCC reference method for pH measurement in blood is based on the use of a cell consisting of a H^+ ion-selective glass electrode and a reference electrode

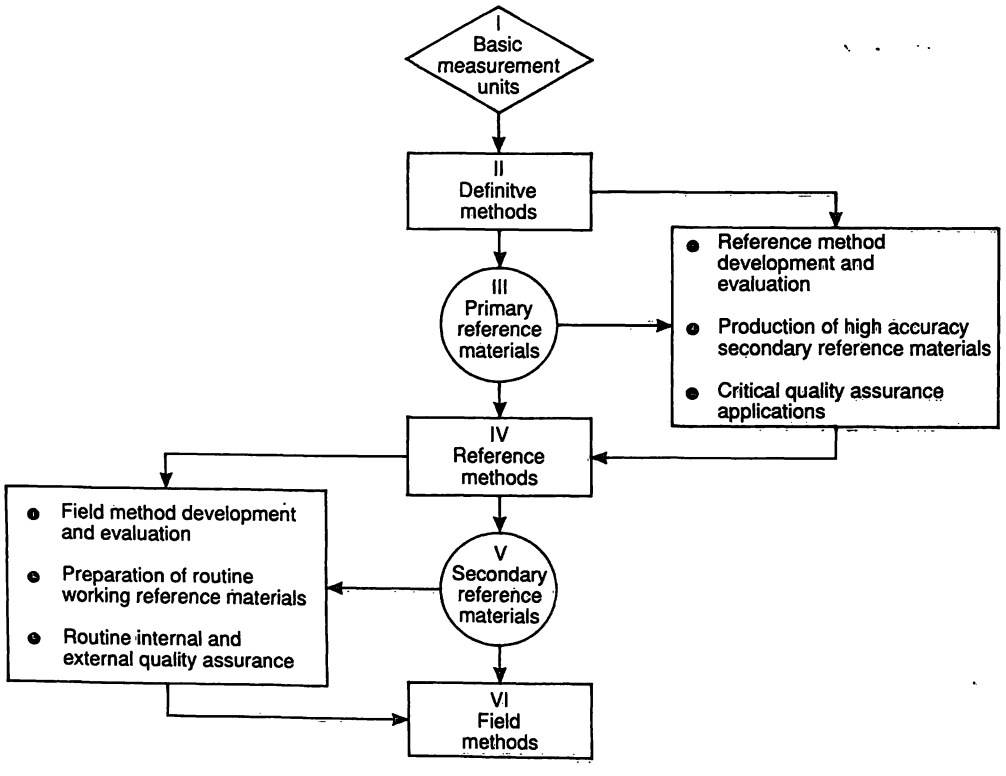

Fig. 1. Schematic presentation of the relationship among the technical components of an "idealized", accuracy-based chemical measurement system.

with a saturated KCl solution as salt bridge according the scheme:

"Ref. Electrode" "Glass Electrode"

R_1 | KCl (satd.) || sol. X | G | inner ref. sol. | R_2 (cell b)

Electrodes R_1 and R_2 are connected to the pH-meter. The whole system is thermostatted at 37 °C.

Two primary calibration solutions of the NBS series are needed for calibration: phosphate buffer of pH = 7.392 (37 °C) is used for setting the zero point of the instrument and the phosphate buffer pH = 6.839 (37 °C) for the slope adjustment. In figure 2 a schematic presentation of the pH measuring system is presented. The glass electrode ought to be a good approximation of the hydrogen electrode. The capillary type is chosen for the reference method of pH measurement in blood. To obtain the true plasma pH a small *bridge of plasma* should be interposed between the blood and the concentrated KCl to avoid the effect of the blood cells minimizing the residual liquid/liquid junction potential.

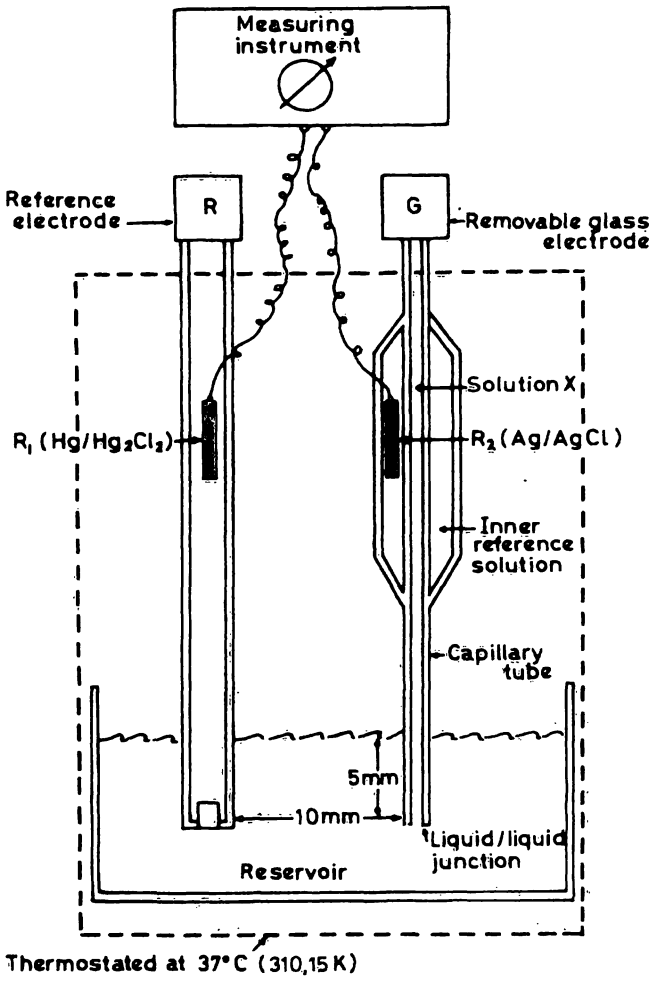

Fig. 2. Schematic presentation of the pH measuring system.

The pH measuring system of the BMS2-Mk2 from Radiometer (Copenhagen, Denmark) is an adequate system which fullfils the requirements of the presented reference method. The pH values of blood samples determined with this system can be directly compared with pH values obtained with the definitive method.

For further details reference should be made to the document for pH measurement which was approved in 1986 by the IFCC (2).

1.2 Tonometry

The reference method for tonometry of blood makes possible the preparation of blood samples with known partial pressure of gases (CO_2 and O_2).

Definition

Tonometry designates the procedure of equilibrating a liquid sample with a gas of known composition under controlled conditions in order to establish a known partial pressure (tension) of a gas in the liquid. The instrument employed is called a tonometer.

Methods of tonometry

Tonometry may be performed in two different ways.

a. Using a *closed system*, a gas and a liquid are brought into contact; after equilibrium the resulting composition of the gas phase is measured. This method is laborious and time-consuming and cannot easily be adopted for routine work.

b. Using an *open system*, a gas mixture of known composition flows through the system generating identical partial pressures of gases in the fluid sample and the gas mixture. In contrast with the closed system this method eliminates gas analysis after tonometry and has been chosen for the *present reference method.*

The requirements of *instrumentation and equipment* are a gas mixture, a humidifier and a tonometer, the latter two being thermostatted at 37 °C.

The *primary gas mixtures* of carbon dioxide, oxygen and nitrogen are gas mixtures prepared gravimetrically according to the definitive method from the constituent gases. The constituent gases should be of sufficiently high purity and not contaminated with one of the other constituents of final mixture. The relative inaccuracy of the composition of the gas mixture i.e. CO_2 and O_2 substance fractions, should be less than $\pm$ 0.3% of the stated value.

For certification of gas mixtures, a service is provided by national institutes and by some industries.

Also gas mixtures prepared from pure gases with calibrated gas mixing pumps meet the requirements.

In the *humidifier* the gas is preheated at 37 ± 0.1 °C, then saturated with water vapour by bubbling through water at the same temperature and the actual barometric pressure, to prevent evaporation or condensation of water in the sample. The gas is admitted to the equilibration chamber of the tonometer without cooling or alteration of pressure.

A *film type tonometer* which should provide a minimum volume of 3 ml of tonometered blood has been chosen for the reference method.

In practice, a *Laue* tonometer (4) from the firm Eschweiler (Kiel, Germany) is, according to our experience, an adequate system. This tonometer is preferable to the IL-tonometer (Instrumentation Laboratory SpA, Milan, Italy) which has a temperature gradient of 0.3 °C between the sample and the waterbath (5). In addition this system needs much more gas mixture (5).

For further details reference should be made to the in 1988 approved IFCC document on tonometry (3).

The tonometered blood samples are *secondary reference materials* which should be employed for quality control when measuring the partial pressure of carbon dioxide and oxygen in blood. This allows both the accuracy and the precision of the instrumental methods to be evaluated. The method may also be applied for determining acid-base and oxygen transport parameters e. g. p_{50}.

2. Electrolytes

There are several positive features of *ion-selective electrodes* that lead to the conclusion that they have important roles to play in clinical chemistry.

1. Ion-selective electrodes sense the electrochemical activity of the ion species in plasma water rather than its substance concentration in whole plasma. The activity in plasma is probably the more important from a biochemical or physiological point of view.
2. Ion-selective electrodes enable a direct measurement of electrolyte levels to be carried out in small samples of undiluted serum, plasma, whole blood, and urine which is a great advantage in paediatric and intensive care where rapid analyses are essential.
3. Ion-selective electrodes enable us to monitor the ions under investigation continuously with extracorporeal devices or with intravascular catheter tip devices in a patient in vivo, for example during the course of an operation.

Consequently, measuring systems with ion-selective electrodes for sodium, potassium, ionized calcium and recently also for lithium became routinely available, but not without problems in their clinical chemical application (6, 7).

Ion-selective electrodes for sodium and potassium ions may be used on two different ways, namely in undiluted or in diluted samples. These determinations have been called repectively *direct* and *indirect* potentiometry, but this terminology is incorrect; in both cases the measurement is performed directly in different samples.

When the sample has been highly *diluted* the total concentration expressed in mmol/l serum or plasma should be measured at the end. The ionic strength of the dilution will be equal to that of the calibrator. This type of measurement with ion-selective electrodes usually gives no problems, in contrast to measurements in undiluted samples.

The problems of measurement in *undiluted* specimens which are arising with the introduction of ion-selective electrodes for sodium, potassium and also calcium ions in the clinical laborabories are:

a. *What should be reported?*

History was largely determined that ions such as sodium are usually measured on prediluted specimens using flame photometry. In principle the flame photometer measures the total substance concentration of the ion and the ion-selective electrode measures the activity of the free ion in the water phase. Hence differences between results have been obtained by several authors. It is therefore necessary to decide whether we want to report activity or substance concentration in either the total volume of plasma (mol/l) or only in the water phase of plasma (mol/l), the latter being equivalent to molality (mol/kg).

b. *Differences in measuring technique*

Different instruments on the market for the measurement of sodium, potassium and ionized calcium by ion-selective electrodes on diluted specimens give different results, indicating a problem of measurement technique i. e. calibration solutions, selectivity and sensitivity of the electrodes, measuring time, liquid junction potential and temperature.

It will be clear that there is a need to standardize the measuring technique, to develop a reference method for ionized calcium and to prepare reference material.

To consider the above-mentioned problems, a *European Working Group on Ion-Selective Electrodes* has been set up by the International Federation of Clinical Chemistry, consisting of Clinical Chemists and manufacturers with a common interest in exchanging ideas and information on ion-selective electrodes and trying to solve the problems by the increasing use of this new technology. Their goal is to gather data concerning the clinical chemical application of ion-selective electrodes and to obtain consensus concerning definitions, terminology, sampling, calibrating of instrumentation, quality control and reference intervals.

The group started in 1982 and has held meetings in Oslo (1983), Oxford (1984), Helsinki (1985), Graz (1986), Danvers (1987), Stresa (1988) and Monterey (1990), in which European, American, Australian and Japanese invited scientists both from profession and industry participated.

2.1 Sodium and potassium

For many years, results of determinations of sodium and potassium ions in physiological fluids have been expressed in terms of the substance concentration of total sodium and potassium ion (mmol/l). Hence the use both of substance concentrations of sodium and potassium and of their reference intervals is firmly established in clinical interpretation and practice. Furthermore, it can be envisaged that analytical systems which measure substance concentration, such as flame photometers, will continue to be used alongside ion-selective electrode determinations in undiluted plasma for the forseeable future. In consequence, the convention proposal represents a pragmatic compromise which attempts to facilitate the introduction of ion-selective determinations of sodium and potassium ion concentrations in whole blood or diluted plasma into clinical practice while minimising the risk of clinical misinterpretation.

A convention for sodium and potassium is proposed whereby, for routine clinical purposes:

A. Results of ion-selective electrode determinations of sodium and potassium in whole blood and undiluted plasma should be reported in terms of concentration (mmol/l).

B. Results of measurements on standard normal specimens should conform exactly with those obtained by flame photometry on the same specimens.

C. Standard plasma specimens are herein defined as having mass concentration of plasma water of 0.93 kg/l, plasma bicarbonate concentration of 24 mmol/l, plasma pH of 7.40, and concentrations of albumin, total protein, cholesterol and triacylglycerol within the reference range for healthy subjects.

For practical purposes, it is satisfactory if the values of the flame photometer and the ion-selective electrode for *normal* plasma are equivalent. This may be achieved in several ways (6, 7):

1. With pooled normal plasma samples.

2. With some spiked samples of which the concentration of sodium or potassium has been increased by adding known amounts of sodium chloride and potassium and decreased by partial ultrafiltration.

3. With a correlation study between ion-selective electrode and flame atomic emission spectrometry of 100—200 sera samples with electrolyte concentration covering the clinical range and fulfilling the normal conditions.

When this convention is used, results reported by ion-selective electrodes in undiluted normal plasma are equivalent to the substance concentration of the ion. However, in samples with normal plasma water mass concentration or with abnormal concentrations of complexes of the ion, results are numerically different from true substance concentration.

Table 1 shows calculated examples of the differences between the substance concentration obtained by flame photometry and the results obtained with ion-selective electrode when the ionic strength or the mass concentration of water varies. When the appropriate conversion factor (1.27 mol/l) is applied, the ion-selective electrodes reading, "c_{tNa}", is the same as the result obtained by flame photometry for normal plasma. With the same factor, small discrepancies are observed when the ionic strength of the plasma varies, because the activity coefficient of Na^+ is dependent on the ionic strength. However, the discrepancies are very small, even with extreme pathological variations of ionic strength, and the discrepancies do not have any practical consequences. Large differences arise only when the water concentration of the plasma changes, for example, because of severe hyper- or hypoproteinaemia or severe hyperlipaemia. In the example where the mass concentration of water has decreased by 15% to 0.80 kg/l, the differences between the flame photometer and the ion-selective electrode results is 20 mmol/l.

Tab. 1. Calculated examples.

Specimen	Flame c_{tNa} mmol/l	Ion-selective electrode $a_{Na^+} \times 10^{-3}$	"c"$_{tNa}$* mmol/l	Difference c_{tNa} − "c"$_{tNa}$ mmol/l
Normal plasma γ_{Na^+} = 0.747 ϱ = 0.933 kg/l I = 0.160 mol/kg	140	110.2	140	0.0
Hypernatraemia γ_{Na^+} = 0.737 ϱ = 0.933 kg/l I = 0.190 mol/kg	170	132.0	167.7	+ 2.3
Hyponatraemia γ_{Na^+} = 0.760 ϱ = 0.939 kg/l I = 0.130 mol/kg	110	88.1	111.9	− 1.9
Hyperlipaemia γ_{Na^+} = 0.747 ϱ = 0.800 kg/l I = 0.160 mol/kg	120	110.2	140	−20.0

* Calculated concentrations obtained by multiplying ion-selective electrode a_{Na^+} values × 1.27.

Physiologically and biochemically, results obtained by ion-selective electrodes are to be preferred because they more accurately reflect the pathophysiological status of these ions in plasma water and are thus more relevant clinically than those reported by flame photometer. Actually, therefore, this is a problem of the flame photometer and not of the ion-selective electrode. It is recommended that results obtained with severely hyperlipaemic sera always be accompanied by an explanatory remark, i.e. that the flame photometer result is spuriously low ("pseudohyponatraemia"), but that the ion-selective electrode result is not affected.

2.2 Calcium

The relationship between ionized calcium and total calcium is complex and variable for each sample. Ionized calcium reflects better the homeostasis of calcium than total calcium, due the dependence of total calcium on proteins and nutrition. Total calcium may underestimate hypercalcaemia and overestimate hypercalcaemia. Therefore ionized calcium is clinically the relevant value.

Convention of reporting ionized calcium

In principle, ionized calcium measurements could be reported as concentration (mmol/l plasma water), molality (mmol/kg plasma water) or as activity. To avoid a proliferation of units, the convention is hereby adopted of reporting ionized calcium measurements as concentration expressed as *moles per liter plasma water and not per liter plasma.* Therefore the measured calcium ion activity should be converted to substance concentration of calcium ion by a factor which is primarily dependent on the reciprocal molal activity coefficient of calcium ion of normal plasma.

For practice the calcium ion-selective electrode should be calibrated in terms of concentration. The composition of the calibration solutions is chosen such that the activity coefficient of the calcium ion is assumed to be identical both in calibration solutions and normal plasma i.e. by convention $I = 0.160$ mol/kg.

The proposed recommendations for *a reference method of ionized calcium* are analogous to pH. Specifications and properties of calcium-selective and reference electrodes, the salt bridge, the cell geometry and millivoltmeter as well as primary and secundary calibration solutions ($I = 0.160$ mol/kg) and the procedure of measurement are provisionally described (8).

A prototype measuring cell system for the reference method for the measurement of ionized calcium in serum, plasma and whole blood is under development by *Covington & Maas*, and will be tested by an international group of scientists.

Preanalytical factors of influence

The concentration of ionized calcium in blood, plasma or serum may be influenced by pH changes of the sample, calcium binding by heparin and dilution by the anticoagulant solution. Therefore, and in view of the increasing interest in the determination of ionized calcium in the clinic, recommendations for optimal conditions for the collection and processing of blood specimens for both routine measurements and for establishing reference ranges are also in preparation.

For users of ion-selective electrodes who wish to express results of ion measurements in terms of activity, values of activity coefficients will be recommended in the near future, thereby providing calculated activity values of sodium, potassium and calcium in a number of specified aqueous solutions which are suitable for instrument calibration.

3. Quality Control

3.1 pH and blood gases

Generally, a quality control material is a stable and homogeneous material which can be amply supplied. One or more properties of it are determined by reference methods. In blood gas and pH analysis, acid-base and oxygen buffering characteristics are most important. Thus good quality control material for pH and blood gases prepared from blood should have normal acid-base properties, oxygen buffer capacity and oxygen haemoglobin (Hb) equilibrium curve. It should also have a low and stable fraction of haemiglobin (Hi). It should not clog tubing or pollute electrode membranes.

The ideal quality control material for blood gas and pH measurements is the sample itself with reference values obtained by using the reference methods for pH and tonometry.

The next best material for quality control purposes in blood gas chemistry is fresh, whole human blood, freed of platelets and leukocytes to reduce the rate of oxygen consumption and carbon dioxide production in the sample. This is accomplished by centrifuging the blood, removing the platelet and leukocyte layer by aspiration and resuspending the erythrocytes in the plasma. The reconstituted blood sample is then equilibrated in a tonometer to establish a known pCO_2 and pO_2. The pH of this tonometered blood must be measured separately, using the IFCC approved (1986) method for pH measurement in blood.

This material is designated a *primary quality control material.*

Storage of this material in a gas-tight syringe in iced water is possible for up to 2 hours (5, 9).

However, the disadvantages of blood are its poor availability, its instability due to metabolism and the fact that it may constitute a biohazard to the user. The inconvenience and limitations of blood use led to substitution with various serum and aqueous preparations as control matrices.

At present, quality control materials are available with matrices that are different from blood. The products are divided into several groups according to composition (2):

1. Aqueous electrolyte solutions.
2. Aqueous electrolyte solution containing ethylene glycol.
3. Aqueous electrolyte solution containing protein.
4. Aqueous electrolyte solution containing fluorocarbon suspension.
5. Aqueous electrolyte solution containing stabilized red blood cells.
6. Frozen haemolyzed red blood cell solution.
7. Aqueous electrolyte solution containing human Hb.

The aqueous materials have advantages such as a long shelf-life and their availability in ampoules which are ready for use. However their disadvantage is poor oxygen buffering, although the fluorocarbon material has an oxygen buffering capacity which is, at a single pO_2, equivalent to that of blood. Moreover, certain problems of pH measurement are not reproduced by aqueous materials without protein.

Stabilized blood preparations have a short shelflife and a poor and changing oxygen buffering capacity, due to methaemoglobin formation. The haemoglobin solution has the disadvantages that values for pCO_2 and oxyhaemoglobin concentration tend to be decreased, while values for pH, pO_2 and methaemoglobin concentration are increased. Oxygen buffering, however, is close to that of blood, although poorer in the physiological pO_2 range of 7 – 20 kPa. Hence, the differences between the control matrix and the patient's blood specimens introduced a new problem: non-identical behaviour.

When the assigned pH, pCO_2 and pO_2 values of these products have been determined using the reference method for pH in blood and the present reference method for tonometry of blood respectively, these materials are designated *secondary quality control materials.* If the assigned pH, pCO_2 and pO_2 values of

these products are established by routine measurements on the blood gas instruments themselves, these products should be designated *tertiary quality control materials* with expected values. These tertiary controls do not allow evaluation of *the accuracy* of the system although *the precision* may be properly assessed.

An ideal solution for quality control of blood gas determinations should contain haemoglobin with a normal O_2 affinity, a normal c_{tHb} and a low x_{Hi}, comparable to that of fresh whole blood. Also the haemoglobin (Hb) solution must be free of erythrocyte stromata and other precipitable matter during storage at 4 °C and use at 37 °C, and it should be easy to prepare. If, in addition to the pH and gas values, the c_{tHb} and the fractions of all Hb-derivatives are known, then such a Hb solution may be suitable for quality control of Hb-meters and CO-oximeters.

Sprokholt (9–13) and *Maas* have described a stable *stroma free haemoglobin solution* which overcomes the disadvantages of whole blood and is sufficiently similar to blood to neglect the matrix differences. Stroma-free haemoglobin solution may be buffered in order to obtain the desired acid-base properties. Stroma-free haemoglobin solution contains functional Hb with the same oxygen buffering capacity as in normal blood; this is achieved by using modulators that alter the oxygen affinity. A suitable solution for pH and blood gas control may be prepared by tonometry with a gas mixture of known CO_2 and O_2 content, and by reading the pH from a pH/log pCO_2 curve previously determined with the reference method for pH. Since protein, Hb, is present, the pH measurement can be properly checked.

The stability of stroma-free haemoglobin solution may be improved greatly by adding NADH, which acts as a substrate of the methaemoglobin-reductase system and keeps the met-Hb (Hi) concentration low.

3.2 Electrolytes

Quality control of ion-selective electrodes has been performed generally with protein-free solutions. However the influence of protein both on the ion-selective electrode itself and on the diffusion potential at the boundary of the salt bridge, as well as the small mV-interval for the whole pathophysiological range of the Na^+- and Ca^{2+}-ion-selective electrode, require the use of protein-containing control material.

The scheme of the liquid junction in figure 3 shows that a protein-free solution is capable of monitoring only the diffusion potential of the calibrator, and that a solution containing protein monitors that of the plasma or serum sample. This has been demonstrated

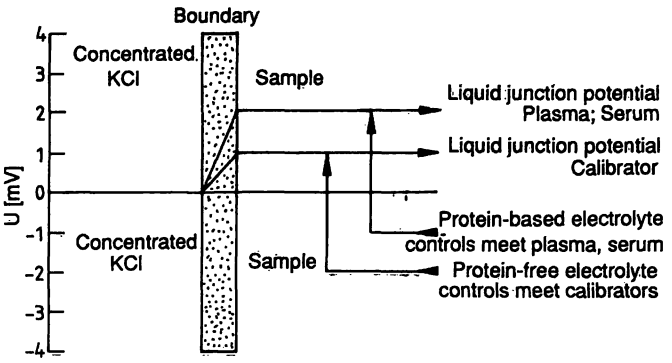

Fig. 3. Schematic presentation of the protein-effect on the liquid junction.

experimentally, and the control results obtained with an analyser for ionized calcium are presented in figure 4 (14). Two protein-free calcium solutions (1 mmol/l and 2 mmol/l) were measured daily and showed constant values. Two serum-based controls measured simultaneously, however, showed a sharp rise in value. Patient or other protein-containing samples would have shown a similar rise. The similarity of the calibrator and the protein-free control solution explains why this liquid junction effect was not observed using the protein-free control solution. This disturbance of the liquid junction occurred because the serum matrix was different. This favours the use of protein-containing solutions in the quality control process of ionized calcium analysers using ion-selective electrodes.

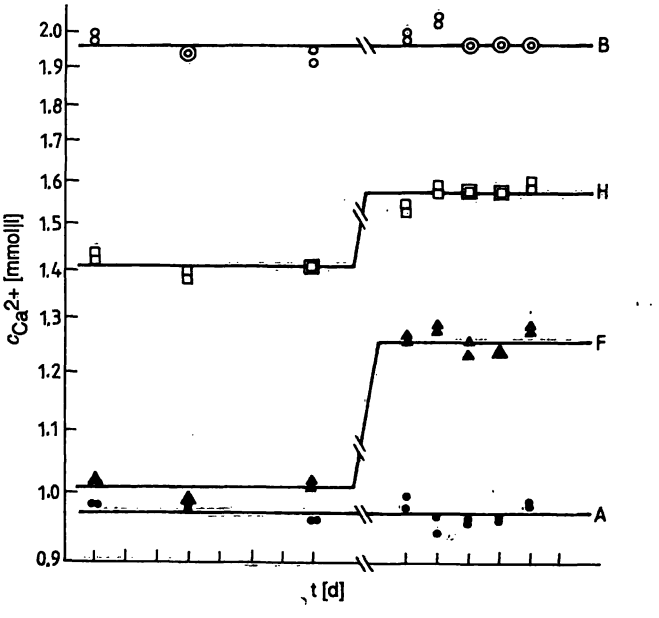

Fig. 4. The concentration of calcium ions ($c_{Ca^{2+}}$) measured on an ionized calcium analyser with protein-free solutions (A and B) and serum-based controls (F and H).

Standard protein-containing reference materials for control of sodium and potassium ion-selective electrodes are under development in the USA by NIST (15). In Japan, Daiichi Pure Chemicals Co., Ltd, in cooperation with the Chemicals Inspection & Testing Institute (CITI), has been manufacturing a freeze-dried serum for control of sodium and potassium ion-selective electrode measurements (16). In the Netherlands, stable bovine albumin-containing controls for pH, blood gases and electrolytes are already commercially available from EURO-TROL B.V. (Wageningen, The Netherlands). In Europe, plans are being made by the Bureau of Reference of the Community at Brussels to standardize the ionized calcium determination in blood plasma by developing both a reference method and protein-containing control material.

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